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# Utilization Of The Water Soluble Fraction Of Wheat Straw As A Plant Nutrient Source

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## Utilization Of The Water Soluble Fraction Of Wheat Straw As A Plant Nutrient Source

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#### **ABSTRACT**

Recovery of water soluble, inorganic nutrients from the inedible portion of wheat was found to be an effective means of recycling nutrients within hydroponic systems. Through aqueous extraction (leaching), 60% of the total inorganic nutrient weight was removed from wheat straw and roots, although the recovery of individual nutrients varied. Leaching also removed about 20% of the total organic carbon from the biomass. In terms of dry weight, the leachate was comprised of approximately 60% organic and 40% inorganic compounds. Direct use of wheat straw leachate in static hydroponic systems had an inhibitory effect on wheat growth, both in the presence and absence of microorganisms. Biological treatment of leachate either with a mixed microbial community or the oyster mushroom Pleurotus ostreatus L., prior to use in hydroponic solutions, significantly reduced both the organic content and the inhibitory effects of the leachate. The inhibitory effects of unprocessed leachate appear to be a result of rapidly acting phytotoxic compounds that are detoxified by microbial activity. Leaching holds considerable promise as a method for nutrient recyling in a Controlled Ecological Life Support System (CELSS).

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#### I. INTRODUCTION

Use of inedible plant residue as a nutrient source in agricultural systems is hindered by the short term reduction of available macronutrients in soil (Alexander, 1978). Elevated organic concentrations in the soil from plant residues cause an increase in microbial growth and concomitant incorporation (immobilization) of available macronutrients. It has been determined that immobilization of nitrogen reduced yields (Fribourg and Bartholomew, 1956, and Munson and Pesek, 1958). Inedible plant residues contain significant quantities of soluble inorganic nutrients (Cummins et al., 1972, and Tukey, 1970). If the inorganic fraction of crop residue could be separated from associated organic constituents, then the use of inedible plant residue in agricultural systems would be promoted. Although leaching does not separate the two components, it is an easy and effective method for extracting large quantities of inorganic nutrients.

Use of leachate would reduce the demand for exogenous nutrients (fertilizers) by encouraging nutrient recycling within agricultural systems. Such a program could be effective for hydroponic culture since the nutrients are already in a water soluble form. Nutrient recycling is of particular importance in a bioregenerative system such as a Controlled Ecological Life Support System (CELSS), currently under development by the National Aeronautics and Space Administration (NASA) for long-term space habitation (MacElroy and Bredt, 1984).

A potential disadvantage of using leachate extracted from a crop is the inhibitory effect of leachate constituents on plant growth. Leachates from several different crops directly inhibited germination and seedling development (Guenzi and McCalla, 1962 and McCalla, 1962). Similarly, leachate from wheat straw inhibited cotton seed germination (Hicks et al., 1989). Most of the total weight loss was in the form of soluble organic compounds (Tukey, 1970). In addition, crop leachate has been shown to promote the production of phytotoxins by certain bacteria (Cochran et al., 1977, and Fredrickson et al., 1988). Pretreatment of leachate to remove or detoxify organic compounds may be neccessary before it can be used as a plant nutrient source.

Biological pretreatment of leachate would convert some organic constituents into microbial biomass (i.e. single cell protein or myco-protein) suitable for use as animal or human food (Rogoff and Rawlins, 1987). The edible oyster mushroom <u>Pleurotus ostreatus</u> L. has been shown to grow well on inedible crop residue (Cazada et al., 1987 and Kahn et al., 1981). This biological conversion of inedible into edible

material has potential for improving the productivity and efficiency of food production in a CELSS.

The initial goal of this research was to measure leaching rates of both organic and inorganic constituents from the inedible fraction of wheat, a CELSS candidate crop (Tibbitts and Alford, 1982). Wheat growth was then compared in leachate-based versus inorganic salt-based hydroponic solutions. The effectiveness of biological pretreatments for reducing the phytotoxicity of leachate was also examined.

#### II. MATERIALS AND METHODS

#### A. Leaching rates

Plant material used for leaching was mature wheat (<u>Triticum aesitivum L. cv.</u>) Yecora Rojo) grown hydroponically in controlled environmental chambers as part of plant growth studies at the Kennedy Space Center, FL. Straw (stems plus leaves) and roots were separated, cut into 25-mm sections, and oven dried at 70 C for 48 h before leaching.

Experiments were conducted in acid-washed, 500-ml glass beakers. Replicate beakers (N = 3 for both straw and roots) contained 5% (w/v) plant material in deionized water (Milli-Q system, Millipore¹) with sodium azide (0.02%) added as a microbial inhibitor. The treatments were incubated at 23 C. Liquid samples were removed after 1, 2, 3, 24, and 96 h, filtered through glass microfiber filters (GF-C, Whatman²), and frozen. Samples were analyzed for NO<sub>3</sub>-N and PO<sub>4</sub>-P by automated colorimetry and for K, Ca, Mg, Fe, Cu, and Zn by inductively coupled plasma (ICP) spectrometric analyses. At each sample time all leached plant material was squeezed by hand and the remaining leachate in the beaker was removed and replaced with an equal volume of fresh deionized water containing sodium azide. Solid residues after 96 h were dried at 70 C for two days and weighed. Subsamples of wheat straw and roots were sent to an accredited nutritional laboratory (Nutrition Int.³).for inorganic analyses

#### B. Plant growth in untreated leachate

Leachate used in the plant growth bioassays was produced by soaking wheat straw and roots (9:1) in water (5% w/v) for 3 h. Leachate was prefiltered (Whatman No 1 filter paper) then sterilized by passage through a 0.22  $\mu$ m pore size membrane

<sup>&</sup>lt;sup>1</sup>Milipore Corp., Bedford, MA

<sup>&</sup>lt;sup>2</sup>Whatman International Ltd. Maidstone, England

<sup>&</sup>lt;sup>3</sup>Nutrition International, East Brunswick, NJ

filter. Wheat plants were grown in solutions containing various concentrations of leachate (3%, 9%, and 20%) and were compared to wheat grown in a control solution consisting of modified 1/2 strength Hoagland #1 solution (Hoagland and Arnon 1938). Solutions were prepared in autoclaved 18-L carboys (Nalgene<sup>4</sup>) by adding appropriate levels of filter-sterilized deionized water, leachate, and inorganic nutrients. Solutions containing leachate were amended with nutrients to approximate concentrations present in the control solution (Table 1). All solutions were adjusted to pH 5.8 ± 0.2 units with 1 M KOH. Microbial inoculum (10 ml) from a functioning hydroponic system containing wheat plants of various ages was added to each carboy. The solutions (treatments) were distributed to sterile 2.2 L Nalgene bottles.

Wheat seeds were surface sterilized using a combination of mercuric chloride and hydroxylamine hydrochloride (Barber, 1967). Seeds were germinated on moist filter paper in sterile petri plates at 23 C for 24 h. Seedlings were transferred to sterile germination boxes (12 per box) modified with vertically positioned inserts used to orient the seedlings. Each insert was covered with polyester, which acted as a wick for the deionized water at the bottom of the box. The roots grew downward along the polyester to the bottom of the boxes. This procedure produced seedlings with straight, vertical roots, which facilitated successful transplantation into the Nalgene bottles. At 4 days, each seedling was wrapped in a sterile foam plug and inserted into a 20 mm hole located in the center of the lid of each bottle. The nutrient solution was bubbled with sterile laboratory air, delivered by an aquarium pump through a 0.22 µm pore size membrane filter cartridge (Millipore) to a gassing manifold. The manifold terminated in a 22 ga. syringe needle that penetrated a serum stopper fitted to a second hole through the bottle cap. Air was delivered to the bottom of each bottle by a pasteur pipette fitted to the inside of the serum stopper.

Plants were grown under high pressure sodium lamps at a PPF of  $300 \, \mu \text{mol m}^{-2} \, \text{s}^{-1}$  for 28 days. Photoperiod was 18/6 h light/dark. Bottles were placed under the lights according to a randomized block design with 4 replicates. Temperature was maintained at  $23 \pm 2$  C and relative humidity at  $60 \pm 10\%$ . Plant growth was determined from oven-dry weights (48 h at 70 C) of the harvested biomass and by final shoot height.

<sup>&</sup>lt;sup>4</sup>Nalge Co. Rochester, NY

#### C. Plant growth in sterile versus nonsterile solutions

Leachate solutions (9% and 20%) were prepared as above. Sterile treatments were administered before the carboys were inoculated with 10 ml of nutrient solution. Samples of the nutrient solution from uninoculated bottles were plated on R2A agar (Difco<sup>5</sup>) after 14 and 28 days to test for sterility. All other experimental conditions and methods were identical to the first study. A randomized block experimental design was used with 4 replicates.

#### D. Plant growth in biologically pretreated leachate

This study compared plant growth in five nutrient solutions: A. modified 1/2-strength Hoagland's, B. modified 1/2-strength Hoagland's with nutrient concentrations similar to 10% leachate, C. 10% leachate, D. 10% leachate pretreated with a mixed microbial community, and E. 10% leachate pretreated with Pleurotus ostreatus L.

All solutions (excluding 1/2 Hoagland's) were adjusted with nutrients so the inorganic concentrations would be similar, as was done in study B (Table 1). Microbial and fungal pretreatments of leachate were conducted for 4 and 7 days, respectively, in 2-L flasks containing 500 ml filter-sterilized leachate. Flasks were shaken at 250 revolutions min<sup>-1</sup> on a rotary shaker at 25 C. Nutrient solution from a recirculating hydroponics system containing actively growing wheat (30-day) was used as the microbial inoculum (1% v/v). Fungal inoculum consisted of mycelia from a 12-day-old culture of P. ostreatus grown on yeast/mannitol broth (Difco). Cultures were initiated with inoculum from slant culture and grown at 25 C and 250 rev min<sup>-1</sup>. Mycelia were washed with phosphate buffered saline

 $(PO_4^- = 0.1 \text{ M}, \text{ saline} = 0.85\%, \text{ and pH} = 7.2 \text{ units})$  and added at the 10% (v/v) concentration. Pretreated leachate was recovered by filtration of cultures through 20-mesh nylon cloth followed by filter sterilization through 0.22  $\mu$ m membrane filters.

Seeds were sterilized, germinated, and transplanted with methods used in study B. Plants were grown in a 1.8 m x 2.4 m walk-in growth chamber (model M48, EGC<sup>6</sup>) under Vita-lite phosphor, fluorescent lighting (250  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> PPF), with a 20/04 h (light/dark) photoperiod. Both temperature (23.0  $\pm$  0.4 C) and relative humidity (65  $\pm$  5%) were held constant .

Along with inorganic analyses of the solution and plant tissue, total organic carbon (TOC) analyses were performed on the solutions prior to and after the experiment by

<sup>&</sup>lt;sup>5</sup>Difco Laboratories, Detroit, MI

<sup>&</sup>lt;sup>6</sup>Environmental Growth Chambers, Chagrin Falls, OH

wet chemical oxidation (model 0524B-HR, Oceanography International Corp<sup>7</sup>). A randomized block (according to harvest date) experimental design was used with 4 replicates. Experimental results were compared using analysis of variance (SAS<sup>8</sup>).

#### III. RESULTS AND DISCUSSION

#### A. Leaching rates

Leaching reduced straw and root dry weight more than 30%. Chemical analysis of tissue before and after leaching showed that a majority of the inorganic constituents were readily soluble under experimental conditions (Table 2). At least 50% of the plant macronutrients analyzed (PO<sub>4</sub>-P, K, Ca, Mg) were released in 96 h (Table 2). Analysis of nitrogen was not done, so the recovery values could not be determined. The recovery of micronutrients was generally lower (27-62%) than for the macronutrients (> 50%). The role of heavy metals as constituents in membrane-bound enzymes and other insoluble protein complexes may be the cause of low recovery in water extracts (Sandman and Bolger 1983, Sedbeck 1982).

Estimates of mass balance from the chemical analyses indicate that organic and inorganic matter constitute 60% and 40%, respectively, of leachate dry weight. TOC data were converted to a dry weight basis assuming that all organic compounds were of the molecular formula CH<sub>2</sub>O. In separate studies, the organic content of leachate was determined to be between 50% - 70%, based on evaporative drying and subsequent combustion. In comparison, the organic content of unleached biomass is typically 90% or more. This makes the dry weight ratio of organic:inorganic materials lower in leachate (6:4) than in the unleached biomass (9:1).

Organic and inorganic material were rapidly released into water from both the straw and root tissues. The majority of total leachable material of both inorganic and organic origin was released within the first 3 hours. The leachable total is defined here as the amount of material released after 96 h. The pattern of release of both inorganic and organic material over time followed a hyperbolic curve with a gradual approach to maximum (i.e. Figs 1a, b, c).

<sup>8</sup>SAS Institute Inc., Cary, NC

<sup>&</sup>lt;sup>7</sup>Oceanography International Corp. College Station, TX

Corresponding to the curve, the amount of material leached can be described by a first-order rate-of-reaction equation (Daniels and Alberty, 1967) having the form:

 $A \rightarrow P$ , where A equals the leachable fraction of wheat biomass, and P equals the amount of leachable material in solution (product). The velocity v can be expressed as follows:

$$\mathbf{v} = \underline{\mathbf{dp}} = \underline{\mathbf{da}} = \mathbf{ka} = \mathbf{k(ao - p)}$$
, where a and p are the concentrations dt dt

at any time t, of A and P respectively.

**ao** = concentration of leachable material at time 0, which also is an estimate of P at time infinity ( $P^{\infty}$ ).

k= first order-rate constant

The equation can be integrated to the form

$$ln(a) = -kt + ln(a0)$$

The plot In (a) vs t (1-3 hr) yields a line with a y-intercept of In (ao) and a slope of -k.

Correlation coefficients of regressions of the plots ranged from 0.80 to 0.98 (Table 3), indicating good fit of the data to the equation. Excluding Fe and Cu, ao was greater for straw. ao (Cu) was similar between straw and root, whereas ao (Fe) was much greater in the root (Table 3). The variations between the nutrient concentrations in straw and root leachates are similar to the variations between composition of straw and root tissue of mature wheat plants grown in previous hydroponic studies (Mackowiak et al., 1989). The k values also are similar between straw and root for most nutrients (Table 3). This suggests that wheat straw and root tissue have similar characteristics in the leaching rate of inorganic nutrients. However, since straw represents approximately 90% of the inedible biomass, it contributes the vast majority of inorganic nutrients to the total leachate nutrient pool.

Although P<sup>∞</sup> is reached more quickly for leachable organics than for inorganic nutrients, the trend is not reflected by the calculated k values, which are lower for organic material than for some inorganic nutrients (Table 3). More frequent sampling

during the first hour of leaching would probably be neccessary for a more accurate calculation of leaching rate of organic material.

Although present estimates of ao and k are useful for relative comparisons of the leaching of nutrients, future experiments should employ different methodologies to more accurately model leaching. For example, k values calculated from these data indicate that more samples should be taken earlier than 1 h. It should be taken into account that the rate reactions of some, if not all, eachable materials may be of greater orders (i.e. 2nd and 3rd orders). Size reduction of the residue or mechanical compression of plant residue at each sampling to fully express residual water or continual sampling over time from a leaching vessel without any water change may yield better estimates of  $P^{\infty}$ .

Results clearly indicate that the majority of inorganic nutrients from wheat residue can be rapidly recovered (2-3 h) using water and minimal energy input. Recovery of the relatively small proportion of nutrients remaining in the straw by altering leaching conditions (i.e., mixing, chemical solvents, heat) may not offset added energy costs needed for such treatments. Use of chemical solvents may require purification or neutralization of leachate. Hot water extractions may increase the solubilization of hemicelluloses, thereby increasing the organic:inorganic ratio if mineral recovery remains unchanged (Cunningham and Carr 1984, Cunningham et al. 1986). However, it has been reported that cold water extracts of several crops contained greater quantities of toxic materials than did hot water extracts (Guenzi and McCalla, 1962).

Substrate preparation, on the other hand, may effectively increase nutrient recovery. Drying plant material has been shown to significantly increase leaching rate (Harrison and Mann 1975, Godshalk and Wetzel 1978). Current studies are underway to evaluate leachate of fresh wheat residue versus residue which has been oven dried at 70 C. Mechanical compression of leached residue would require some energy, but it may increase nutrient recovery by expressing residual water. Grinding the residue prior to leaching, which may increase the recovery of nutrients, may also require mechanical compression as a seperation method since filtration would be difficult and costly.

#### B. Plant growth in untreated leachate:

The relative concentrations of inorganic nutrients in the leachate are not equivalent to those in a defined inorganic medium such as Hoagland's solution. The concentration of iron is particularly low, while those of Cu, Mn, and Zn are disproportionately high (Table 1). A 3% leachate solution, for example, would

require significant inorganic nutrient amendments, but would not contain toxic concentrations of any nutrients. Nutrient solutions containing higher concentrations of leachate would require less amendments, but may contain excessive amounts of trace metals. However, the pH of the leachate solution (5.7 - 6.7 units) is conducive for direct use in hydroponic solutions.

Plant growth data indicated that the presence of leachate in nutrient solution affected plant growth negatively, even when inorganic nutrient concentrations were normalized (Fig 2). Biomass of plants grown in solutions containing leachate was significantly less (P=0.05) than that of plants grown in a defined inorganic medium (Hoagland's). Reductions in biomass were more severe at higher leachate concentrations, particularly at 20%, where plant biomass was only 20% of the control.

While nutrient imbalance in leachate-based solutions may play a role in inhibiting plant growth at high leachate concentrations, it is much less likely to cause inhibition at lower leachate concentrations (e.g. 3%). Toxic concentrations of non assayed metals (i.e. Cd, Cr, Pb, Ni) also areunlikely since subsequent studies have found insignificant levels of these elements in leachate (unpubl. data). The observed dose-response pattern of reduced plant growth with increasing leachate concentration suggests the presence of phytotoxic compounds in the leachate.

#### C. Plant growth in sterile versus nonsterile solutions:

Solution sterility was confirmed when samples from uninoculated treatments tested negative for bacteria-forming colonies when plated on R2A agar (Difco<sup>5)</sup> medium. Inoculation had a negative effect on plant growth in 20% leachate (Table 4), but the magnitude of this effect is minor when compared to the decrease in plant growth in 3% vs. 20% leachate solutions (Table 4). The slight pytotoxic effects of inoculated treatments could be caused by 2 factors; 1. microbial transformation of leachate organics into pytotoxic compounds, 2. microbial infection of stressed roots. Phytotoxicity of native leachate organics is greater than the deleterious effects caused by microorganisms.

#### D. Plant growth in biologically pretreated leachate:

Results from the leachate pretreatment experiment support the hypothesis that the organic fraction of leachate directly inhibits plant growth. Plant growth in modified Hoagland's solution with inorganic salts added to match the concentrations found in a solution with 10% leachate produced as much biomass as a normal Hoagland's solution (Fig 3). Once again this indicates that inorganic nutrient imbalances were not the primary cause of plant growth inhibition.

Pretreatment with the mixed microbial community and <u>P. ostreatus</u> caused significant reductions (50-60%) in the organic carbon content of the leachate (Fig 4). Plants grown in these biologically pretreated leachates produced significantly greater biomass after 28 days than plants grown in untreated leachate (Fig 3). The results indicate that the organic fraction of wheat leachate contains significant concentrations of one or more compounds toxic to wheat growth. These compounds are degraded or detoxified relatively quickly by microbial activity. Given this lability, the compounds probably do not persist in the leachate. In fact, there was a 70% reduction in the organic concentration (TOC) of nontreated vessels after 28 days (Fig 4). The reduced plant growth in these vessels suggests that the phytotoxic effect of leachate organics occurred soon after transplantation. Young seedlings have been shown to be particularly susceptible to phytotoxic leachate (Guenzi and McCalla, 1962), and McCalla, 1962).

In recent studies using an open, recirculating, leachate-based hydroponic system, wheat yields from a 10% leachate solution (with inorganic amendments) were similar tothose from a 1/2 strength Hoagland's solution (Mackowiak, unpublished data). During the 4-day germination period (seedlings were isolated from the nutrient solution), the tanks containing leachate appeared turbid, indicating high microbial densities. By the third day, the solution had become visibly clearer, suggesting that the bulk of labile carbon may have been mineralized. Studies are currently underway to test this hypothesis.

Organic matter decomposition may be enhanced in these well-aerated, recirculating hydroponics systems compared to the stationary, "closed" vessels used in the aforementioned studies. Enhanced microbial activity within recirculating systems during seed germination, when the seedlings have not yet come into contact with the hydroponic solution, may allow for effective biological detoxification of the leachate-based solution. However, there is an added benefit of using a separate biological pretreatment of leachate for microbial biomass production. Biological pretreatment with the edible fungus P. ostreatus holds particular promise for food production from leachate organics.

#### IV. CONCLUSIONS

Biologically treated leachate appears to be an effective part of an integrated system that utilizes inedible crop residue. Preliminary work has shown that removal of the water soluble fraction of crop residue increases the efficiency of subsequent enzymatic conversion of insoluble plant polymers, e.g., cellulose, hemicellulose

(Garland, unpubl. data). The monosaccharides produced from enzymatic conversion can be used to produce human food or animal feed (Rogoff and Rawlins, 1987). Biological pretreatment of leachate, particularly with edible fungi, would add to the food production pool. In addition, the biological pretreatment reduces the toxic effects of leachate, allowing for recycling of the leachate's inorganic nutrients back into the hydroponic system. The increase in efficiency of both nutrient recycling and food production as a result of this approach is particularly important for an intensive bioregenerative system such as CELSS.

Table 1. Composition of nutrient solutions used in preliminary studies B and C.

		3% Leachate		10% Leachate		20% Leachate	
Element	Control <sup>z</sup>	Leachatey	amendment <sup>x</sup>	Leachatey	amendment <sup>X</sup>	Leachate <sup>y</sup>	amendment <sup>X</sup>
[mM]							•
NO <sub>3</sub> -N	7.50	0.75	7.00	2.25	4.75	5.00	2.14
PO <sub>4</sub> -P	0.50	0.40	0.10	1.31	0.00	2.92	0.00
K	3.00	1.90	1.35	5.73	0.75	12.70	0.00
Ca	2.50	0.25	2.50	0.76	2.00	1.68	1.07
Mg	1.00	0.20	0.80	0.60	0.40	1.34	0.00
s	1.00	?	0.00	?	0.00	?	0.00
[μ <b>M</b> ]							
Fe	0.10*	1.80	0.10*	5.40	0.10*	12.00	88.00
Mn	3.70	2.61	1.10	0.93	0.00	2.08	0.00
Zn	0.32	0.31	0.00	0.93	0.00	2.08	0.00
Cu	0.13	0.12	0.00	0.35	0.00	0.79	0.00
В	0.20	?	0.00	?	0.00	?	0.00
<u>Mo</u>	0.004	?	0.00	?	0.00	?	0.00

Sulphur, boron, and molybdenum concentrations were not determined.

<sup>&</sup>lt;sup>Z</sup>modified 1/2 strength Hoagland's solution #1 Yamount contributed by leachate

Xamount added as inorganic salts \*Concentration in mM

Table 2. Compositional analysis of leached wheat biomass (straw:roots = 9:1) that has been dried at 70 C for 48 h.

Element	Unleached biomass (mg·kg <sup>-1</sup> )	Leached biomass (mg·kg <sup>-1</sup> )	Recovery <sup>z</sup> (%)
P	9200	3860	58
K	62700	13600	78
Ca	10600	5240	51
Mg	3330	988	70
Fe	267	122	54
Cu	33	24	27
Zn	26	10	62
Mn	194	128	34

Sulphur, boron, and molybdenum concentrations were not determined. Zrecovery = 100 x 1- (leached biomass/unleached biomass)

Table 3. Leaching rates: total amount of leachable components (a<sub>o</sub>), first order rate constant (k), and regression coefficients of ln a vs. time.

	STRAW				ROOT			
Element	<b>a</b> o (mg·g tissue <sup>-1</sup> )	k (h <sup>-1</sup> )	r <sup>2</sup>	<b>a</b> o (mg·g tissue <sup>-1</sup> )	<b>k</b> (h <sup>-1</sup> )	r <sup>2</sup>		
N03-N	13.84	1.59	0.92	15.74	1.21	0.96		
PO <sub>4</sub> -P	11.13	0.53	0.98	7.69	0.69	0.98		
K	77.26	0.80	0.97	70.88	0.69	0.94		
Ca	7.20	0.43	0.95	3.82	0.48	0.83		
Mg	4.58	0.61	0.96	1.28	0.60	0.94		
Fe	0.09	0.41	0.80	0.19	0.44	0.86		
Cu	0.01	0.46	0.73	0.01	0.56	0.80		
Mn	0.19	0.36	0.94	0.06	0.53	0.89		
Zn	0.02	0.61	0.97	0.01	0.62	0.84		
TOC	74.72	0.62	0.82	62.97	Z	Z		

ZLeaching rate was too rapid to determine these values.

Table 4. Effect of microorganisms on plant growth at 28 days.

Treatment	Height (mm)	Shoot dwt (g)	Root dwt
1/2 Strength Hoagland's			
Sterile	403 ± 19	$3.05 \pm 0.35$	$0.66 \pm 0.14$
Inoculated	403 ± 29	2.22 ± 0.42	0.54 ± 0.09
20% Leachate			
Sterile	298 ± 36	$0.66 \pm 0.24$	0.20 ± 0.05
Inoculated	168 ± 22	0.09 ± 0.02	0.03 ± 0.00

Means are composed of 4 replicates  $\pm$  standard deviations. Analysis of variance showed significance for leachate concentration and sterility (P < 0.5)

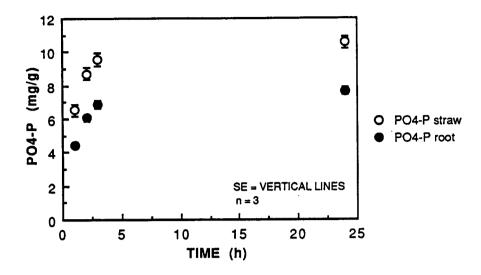


Fig 1a. Leaching of phosphate from wheat biomass.

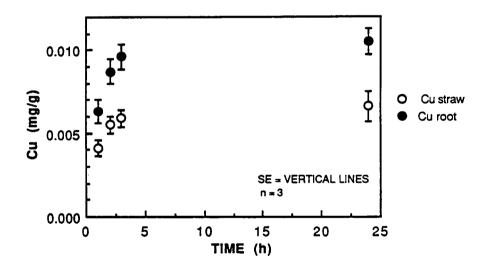


Fig 1b. Leaching of copper from wheat biomass.

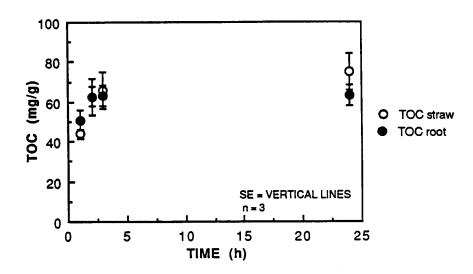


Fig 1c. Leaching of TOC from wheat biomass.

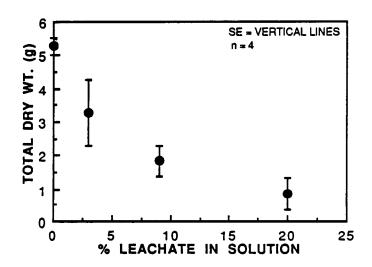


Fig 2. Effect of untreated leachate on plant biomass (28 days).

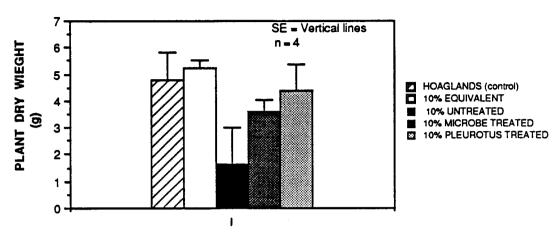


Fig 3. Effect of biological leachate pretreatment on plant biomass (28 days). The 10% equivalent treatment, containing concentrations of inorganic elements similar to 10% leachate treatments, had as much biomass as the control.

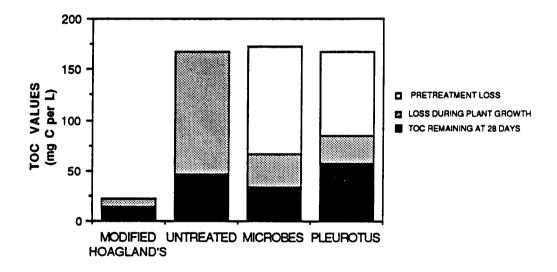


Fig 4. TOC budget of 10% leachate treatments. All treatments contained Fe-EDTA, which accounted for approximately 20-30 mg L<sup>-1</sup> of TOC.

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Recovery of water soluble, i be an effective means of reextraction (leaching), 60% straw and roots, although the about 20% of the total organ was comprised of approximate wheat straw leachate in state both in the presence and about a mixed microbial commin hydroponic solutions, sie effects of the leachate. The	ecycling nutrients we of the total inorgar in recovery of individual carbon from the ately 60% organic atic hydroponic systems are of microorgan in the oyster gnificantly reduced inhibitory effects of the carbon in the carbon	rithin hydroponic nutrient weight dual nutrients valued biomass. In terend 40% inorgalems had an inhinisms. Biologic mushroom Pleuboth the organif unprocessed in	systems. Thresht was removed aried. Leaching ms of dry weigh nic compounds bitory effect on al treatment of rotus ostreatus ic content and eachate appear	ough aqueous ed from wheat also removed at, the leachate Direct use of wheat growth, leachate either L., prior to use the inhibitory robe a result			
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